



Inheritance of resistance to flumethrin in the Mexican Aldama strain of the cattle tick *Boophilus microplus* (Acari: Ixodidae)

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Abstract. The objective of this study was to evaluate the inheritance mode of resistance to flumethrin in the Mexican Aldama *Boophilus microplus* strain. Two Mexican strains were used, the Chiapas susceptible (SS), and the Aldama flumethrin-resistant from Tamaulipas. Six steers weighing ca. 250 kg were randomly assigned for each of six crosses: the susceptible (SS), resistant (RR), and the F₁ (RS, SR) reciprocal crosses and F₂ (RS × RS and SR × SR). The reciprocal crosses were made to evaluate maternal and sex linkage effects. Bioassays tested resistant and susceptible larvae along with their hybrid F₁ and F₂ progeny against a series of concentrations of flumethrin (0, 0.0075, 0.00150, 0.00300, 0.00600 and 0.01200 µg/g). To test the single-gene hypothesis of resistance, a nonparametric line-cross test proposed by Collins was used. The bioassay data were subjected to probit analysis and the resistance factor and effective dominance obtained. Results of this study indicated that inheritance for flumethrin resistance in the Aldama strain was autosomal and controlled for more than one gene. The F₁ and F₂ larvae had similar lower resistant factor (RF 2.8–4.5) while the resistant Aldama strain was 21-fold higher (RF 81.8) than the mean of the F₁ and F₂. The extent of flumethrin resistance in the Aldama *B. microplus* strain depended upon the concentration of the pesticide used. Resistance was almost dominant at the lowest dose while almost completely recessive at the highest dose. Maternal effects were shown for egg-mass. These results shown here indicate more than one gene basis of flumethrin resistance in *B. microplus* ticks are present. Therefore it is necessary to locate and understand the major loci for elucidate the mechanism of resistance and improve the ability to track and delay the evolution of resistance.

Introduction

The cattle tick *Boophilus microplus* (Canestrini) (Acari: Ixodidae) is endemic in tropical and subtropical zones of the world (Estrada-Peña 1999) and results in serious economic losses to animal production. In high yield dairy cows, for example, tick-free cows produced 2.86 l/day more and gained 10.6 kg more than did tick-infested cows (Jonsson et al. 1998). Implementation of control and eradication programs with pesticides has historically been followed by the development of chemical resistance. Organophosphate resistance in *B. microplus* was reported in central and eastern Mexico in 1984. Shortly thereafter, pyrethroids were

introduced and resistance to these chemicals quickly developed (Santamaría and Fragoso 1994).

Successful management of insecticide resistance depends on a thorough knowledge of its genetic basis and the mechanisms involved. The goal of resistance management is to delay resistance in pests (National Research Council 1986). The mode of inheritance helps in resistance detection, monitoring, modeling, and risk assessment. For example, the widely recommended refuge/high-dose strategy is based on the idea that refuges from exposure to pesticide permit susceptible insects to survive and mate with resistant insects emerging from nearby treated crops. This strategy is expected to work best if resistance is caused by a recessive allele so that hybrid offspring from resistant and susceptible parents are killed by the pesticide application (Tabashnik et al. 1997).

Insecticide resistance mechanisms have a biochemical basis. The two major forms of biochemical resistance are target-site resistance, which occurs when the insecticide no longer binds to its target; and detoxification enzyme-based resistance, when enhanced levels or modified activities of esterases, oxidases, or glutathione S-transferases (GST), through increased metabolism of the insecticide, decreased its concentration at the target site of action (Brogdon and McAllister 1998).

Two patterns of pyrethroid resistance were characterized from *B. microplus*. One was knockdown resistance *kdr* mutation is an important mechanism by which ticks develop resistance to pyrethroids and DDT, this mechanism is thought to result from the modification in the sodium channel gene that affects the binding of pyrethroids (He et al. 1999) and the other involved esterase and cytochrome p450 enzyme systems (Miller et al. 1999). *B. microplus* shows genetic variation among strains, two populations (the Gonzalez strain and the Coatzacoalcas strain), have metabolic esterase-mediated pyrethroid resistance; two other populations (the Corrales and San Felipe strains) have target site insensitivity mechanisms for pyrethroid resistance (Guerrero et al. 2001).

Despite the frequent occurrence and economic importance of pesticide resistance in insects, it has not always been possible to clearly identify and characterize its genetic basis. Three questions, the number of genes responsible for resistance, the degree to which resistance to multiple classes of compounds is the result of common mechanisms, and the identification of resistance genes have sometimes proven difficult to demonstrate by definitive analysis (Cochrane et al. 1998).

Tabashnik et al. (1997) reported that populations of *Plutella xylostella* showed variation in the genetic and biochemical basis of resistance to Bt (*Bacillus thuringiensis*) toxins, and two of them shared a genetic locus at which a recessive mutation associated with reduced toxin binding conferred extremely high resistance to four Bt toxins. The other population, in contrast, showed multilocus control.

Similar result was seen in *Drosophila simulans*, where resistance to malathion and permethrin was due to the actions of multiple genes, while resistance to carbamate was largely determined by a single major gene (Cochrane et al. 1998). In *B. microplus* is not known how many genes can cause resistance. The esterase data and cytochrome P450s suggested that many genes produced the same type of resistance (Scott 1995).

The objective of this study was to determine for the existence of a major gene conferring resistance to flumethrin in a Mexican Aldama *B. microplus* strain.

Materials and methods

Tick strains

Two strains of *B. microplus* were used to analyze the genetic basis of resistance. The susceptible strain (SS) was isolated from a field population in Chiapas. The resistant Aldama *B. microplus* strain originally was isolated from a field flumethrin resistant population in Tamaulipas, Mexico that had evolved resistance in response to intensive treatments with flumethrin, and had been reared in the laboratory for nine generations to remove susceptible individuals. This strain was under pressure with flumethrin, in order to maintain the resistance. The LC_{50} was determined in each generation and used as dose for selection pressure of the next generation. The larvae survivors were reinfested on one bovine.

Acaricide

The acaricide used for the larval packet test was flumethrin, a second-generation pyrethroid (Solomon 1983). This pesticide was chosen because prior trials using the Aldama strain showed resistance to flumethrin but not to cypermethrin, deltamethrin or coumaphos. Technical grade (58.78% pure) flumethrin was used to formulate the various dosages. A master stock solution was prepared by dissolving the acaricide in a mixture of olive oil (primary solvent) and trichloroethylene (volatile solvent) at a 1:2 ratio. The high dose used in each bioassay was prepared by diluting the master stock solution with the appropriate volume of the olive oil and trichloroethylene mixture, and then each subsequently lower dose was prepared by serial dilution with the olive oil-trichloroethylene mixture.

Crosses

A total of six steers weighing ca. 250 kg each, were randomly assigned and infested with larvae from the susceptible (SS), resistant (RR), the reciprocal F_1 's (RS, SR) and the F_2 's ($RS \times RS$ and $SR \times SR$) (Table 1). The reciprocal crosses were made to evaluate maternal and sex linkage effects. In order to analyze the six strains at the same time crosses were performed as follows.

First stage

Two steers were infested each with 1 g larvae (ca. 20,000) (De Campos 1998) from the Aldama and Chiapas strains with a camelhair brush on the midline of the back

Table 1. Crosses and reciprocal crosses with the Aldama and Chiapas strains of *Boophilus microplus*.

Strain	Crosses	
	Male	Female
Aldama (Resistant) RR	R	R
Chiapas (Susceptible) SS	S	S
F ₁ RS ^a	R	S
F ₁ SR ^b	S	R
F ₂ RS × RS ^c	RS	RS
F ₂ SR × SR ^d	SR	SR

^aF₁ progeny of mass crosses between 203 males RR with 414 females SS (2.06:1 rate) (RS).

^bF₁ progeny of mass crosses between 193 males SS with 388 females RR (2.01:1 rate) (SR).

^cF₂ progeny of mass crosses between F₁ RS males with F₁ RS females.

^dF₂ progeny of mass crosses between F₁ SR males with F₁ SR females.

of each animal. Larvae used in the infestation were 21 days old at the time of infestation. Each calf was individually stanchioned and held in a covered, open-sided barn. Seven days later metanymphs were collected from each strain. Ticks was placed in 9 cm diameter glass petri dishes and placed in an incubator at $25 \pm 2^\circ\text{C}$, 90% RH until molting to adult. They were then sexed and the reciprocal crosses between adults from each of the strains performed. We determined the sex of each tick visually with the aid of a dissection microscope. Cross-mating between 204 males RR with 464 females SS (2.27:1 rate) (RS) and 226 males SS with 494 females RR (2.18:1 rate) (SR) were performed for 8 days consecutively.

These offspring were transferred to two different steers that previously were placed in a cloth sleeve which had an approximate 20 cm diameter on each side of the body in the dorsal part between the first and sixth rib, in order to reduce the infestation area.

The skin area of 20 cm diameter had been previously shaven with a razor at approximately 3 cm in the perimeter of the circle; subsequently the skin of the animal was impregnated with Resistol 5000[®] and the borders of the cloth were contacted at the points were glued in the shaved area. Finally, the sleeves were closed with rubber bands.

Eight days after the infestation with adult crosses, the engorged female ticks from the four animals were collected for the next 9 days. The engorged females were allowed to ovoposit individually in polycarbonate-covered plates with plastic lids with 24 wells and incubated at $28 \pm 2^\circ\text{C}$, 90% RH for the following 20 days. Twenty days following collection the individual egg masses from 48 randomly selected females from each genetic group were placed in 5 ml glass shell vials stopped up with cotton. Later, the females were separated and weighed. The eggs

were held until hatch began, and the larvae were held for 21 days, at which time they were used for the second stage.

Second stage

Four steers were each infested with 1 g larvae (ca. 20,000) (De Campos 1998) from the Aldama and Chiapas strains and their F_1 reciprocal crosses (RS and SR strains) with a camelhair brush along the midline of the back of each animal. Larvae used in the infestation were 21 days old at the time of infestation. Each calf was individually stanchioned and held in a covered open-sided barn. Seven days later metanymphs from the SS and RR strains were collected. Ticks were placed in 9-cm diameter glass petri dishes and placed in an incubator at $25 \pm 2^\circ\text{C}$, 90% RH until their molt to adults. They were then sexed and the reciprocal crosses between adults from each of the strains were performed.

Cross mating of reciprocal F_1 's between 203 males RR with 414 females SS (2.06:1 rate) (RS) and 193 males SS with 388 females RR (2.01:1 rate) (SR) were performed for 8 days consecutively. These crosses were transferred to two different steers using the same procedure used in the first stage.

Eight days after the infestation with unfed tick adult crosses, we proceeded to collect the engorged female ticks from the six animals each with RR, SS, RS, SR, $\text{RR} \times \text{RS}$ and $\text{SR} \times \text{SR}$ strains, following the procedure described above in stage 1. The eggs were held until hatch began, and the larvae held for 7–14 days, at which time they were challenged with flumethrin using the FAO larval packet test (Stone and Haydock 1962; Anonymous 1971).

Bioassays

Bioassays with resistant, susceptible larvae, and their hybrid F_1 and F_2 progeny were tested against a series of five concentrations of flumethrin (0, 0.0075, 0.00150, 0.00300, 0.00600 and 0.01200 $\mu\text{g/g}$) as follows: Two replicates of each dose, along with a control group containing only the olive oil–trichloroethylene mixture, were made for each bioassay. Treatment papers were Whatman No 1 filter papers cut into 9 cm by 8 cm rectangles. Each paper coded with the appropriate dose and replicate was impregnated with 1 ml of the appropriate dose using a 1 ml pipette to evenly disperse the test solution onto the paper. Using a single Bulldog clamp, each paper was placed on a horizontal rack and allowed to air dry for 2 h so that the volatile solvent would completely evaporate, leaving only the olive oil–acaricide mixture. Once the papers had dried, they were folded in the center and a Bulldog clamp placed on each edge, thus forming a packet. Approximately 100 larvae of 14 days old were placed in each packet using a camelhair brush. To prevent contamination, the brush containing the larvae was not allowed to touch the packet. Different brushes were used for each of the

different strains. The larvae were dropped directly into the packet by taping the brush with masking-tape rod. Once the larvae were added, the open edge was sealed with an additional Bulldog clamp, thus forming enclosed envelope. The treated envelopes were placed individually on a horizontal rack and placed in an incubator at $25 \pm 2^\circ\text{C}$, 90% RH for 24 h. At the end of the 24 h exposure period, packets were opened and mortality counts made. All active, crawling larvae were trapped with the sticky side of a piece of masking tape and counted with a hand counter. The larvae resting in the paper (dead larvae) were closely examined by direct stimulation (either using breath or gently prodded with the end of a pencil) and if they were able to crawl they were counted as alive; otherwise, they were designated as being dead. We recorded for each strain the alive and dead larvae for all the tested dosages.

For the count of hatched eggs, the remainder of the egg-masses in the vials were frozen and homogenized, approximately 100 eggs were sampled and the hatched and unhatched eggs counted by visual inspection with help of a dissecting microscope. In this manner the percentage of hatchability of each egg mass in each strain was estimated.

Data analysis

To test the single gene hypothesis of resistance, a nonparametric line-cross test proposed by Collins (1967) was used. This method requires no assumptions regarding epistatic interaction, normality, distributional equivalence, homogeneity of variance, or optimal scale. So analysis in qualitative characters in crosses of two inbred lines may be performed on untransformed data. If phenotypic differences reflect genotypic variation, the formulas yield accurate Mendelian predictions of phenotypic distributions for segregating generations subject to sampling variation, experimental design considerations and equal viability of genotypes.

The expected values for mortality under the hypothesis of single-locus were calculated for the F_2 's for each of the doses in the following manner:

$$E(p_i F_2) = \frac{NF_2[p_i P_1 + 2p_i F_1 + p_i P_2]}{4}$$

With the variable dichotomy $i = 0, 1$; $p_i P_1$ and $p_i P_2$ the proportions of the parental 1 (resistant) and 2 (susceptible); $p_i F_1$ the proportions of the hybrids; NF_2 the total of individuals in F_2 .

With the obtained values a contingency table was constructed:

$E(p_i F_2)$	$O(p_i F_2)$
$E(p_i F_2)$	$O(p_i F_2)$

and the value of the test statistic was calculated:

$$\chi^2 = \sum_{i=1}^4 \frac{(O(p_i F_2) - E(p_i F_2))^2}{E(p_i F_2)}$$

which is distributed like Chi-square with 1 df.

The laboratory bioassay data were subjected to probit analysis (Robertson and Preisler 1991) with the SPSS package Version 9.0 that involves probit transformation of percentage mortality and \log_{10} (dose) transformation (Finney 1964; Thorne, 1995) to establish the LC_{50} and the LC_{99} values, along with the 95% confidence limits (CL 95%), and the width of the limits was calculated in order to establish the precision of the LC values. Mortality lines were considered identical when their parallelism was not rejected at the $P > 0.05$ probability level. Once the lethal concentration of each strain was determined, the resistance factor was calculate by dividing the LC_{50} value in RR, RS, SR, $RS \times RS$ and $SR \times SR$ by the LC_{50} of SS susceptible Chiapas strain used as a baseline value.

Effective dominance was obtained for resistance to flumethrin in the Aldama *B. microplus* strain. The effective dominance assesses the relative mortality level (ML) for a given insecticide concentration. $D_{ML} = (ML_{RS} - ML_{SS}) / (ML_{RR} - ML_{SS})$. D_{ML} varies between 0 and 1 (0 = survival is recessive and 1 = survival is dominant) (Bourguet et al. 2000).

The individual weights of egg-masses were analyzed by one-way analysis of variance and Tukey test performed. The same procedure was applied to hatchability percentage but transformed to $\arcsin \sqrt{p}$ in order to obtain normality and variance homogeneity; normality tests were performed for this variables (Montgomery 1991).

Results

Resistance in the Aldama resistant strain of *B. microplus* to flumethrin was controlled by more than one gene. The P values resulting from the Chi-square estimators, rejected the single-gene hypothesis, except for 0.006 dose ($P = 0.301$) (Table 2). The LC_{50} in the reciprocal crosses RS and SR was similar (0.004 and 0.0031 ug/g, respectively), as well as their slopes (2.69 vs. 2.10) ($P > 0.05$), thus sex linkage or maternal effects were not evident. Bioassays of F_1 progeny crosses between RR and SS showed that at high concentrations mortality of F_1 and F_2 progeny seem more like the susceptible strain. Conversely, at lower concentrations they seem more like resistant strains. In the middle of the graph line, mortality was intermediate with parental (Figure 1). Table 3 shows the results for the effective dominance of the D_{ML} values with the additive dominance ($D_{ML} = 0.594$) for the commercial dose (0.003), while it was almost recessive (0.1376) at the higher dose and conversely, in the lower dose was nearly dominant (0.874).

Dispersion of individual weight egg-masses produced by pooling the different strains of 193 *B. microplus* female ticks are shown in Figure 2 and shows a normal distribution.

Table 2. Expected values obtained by the methodology of Collins (1967) and observed values, and Chi-square and its P values testing the one-gene hypothesis resistance basis for each dose in Aldama resistant *Boophilus microplus* strain.

Doses ($\mu\text{g/g}$)	Observed F_2 values						
	Survival	Mortality	%Mortality	$E(P_1F_2)^a$	$E(P_0F_2)^a$	χ^2 ^b	P value
0.01200	217	636	74.5	250.17	602.83	6.22	1.20×10^{-3}
0.00600	446	644	59.0	429.32	660.68	1.07	3.01×10^{-1}
0.00300	714	271	27.5	581.16	403.84	74.07	7.55×10^{-18}
0.00150	754	175	18.8	641.01	287.99	64.25	1.09×10^{-15}
0.00075	965	47	4.6	919.33	92.67	24.78	6.43×10^{-7}
0	486	4	0.8	464.35	25.65	19.29	1.12×10^{-5}

^aExpected values: $E(p_iF_2) = NF_2 [p_iP_1 + 2p_iF_1 + p_iP_2]/4$ were $i = 0, 1$. p_iP_1 and p_iP_2 : the proportions of the parental 1 (resistant) and 2 (susceptible). p_iF_1 : the proportions of the hybrids. NF_2 : the total of individuals in F_2 .

^b $\chi^2 = \sum_{i=1}^4 (O(p_iF_2) - E(p_iF_2))^2 / E(p_iF_2) \approx \chi^2$.

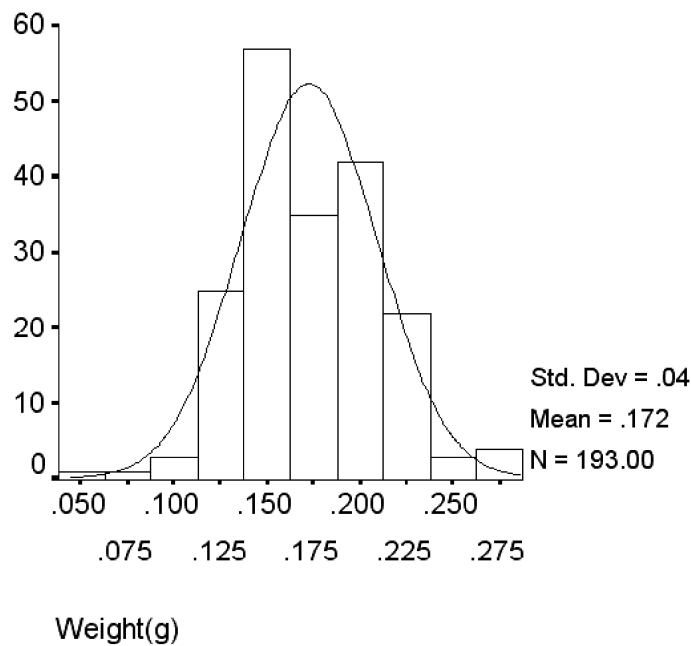


Figure 1. Dispersion of individual egg-masses weights produced by female Aldama *B. microplus* ticks.

Average weights and percentage hatchability for susceptible, resistant strains and their reciprocal crosses are summarized in Table 4. The main differences in egg-mass were due the female strain. Susceptible females and their crosses (RS) had

Table 3. LC₅₀, LC₉₉ values (µg/g), 95% confidence limits (for LC₅₀), slopes and resistance factor using flumethrin against a resistant (Aldama), susceptible (Chiapas), their F₁ reciprocal crosses and F₂ strains of *B. microplus*.

Strain	LC ₅₀ ^a (µg/g)	95% confidence limits for LC ₅₀	Width of 95% confidence limits (for LC ₅₀) ^b	LC ₉₉ ^a	Slope	$\chi^2_{(8)}$	P value	Resistance factor ^c
Aldama RR	0.0900	0.0410–0.5970	0.5560	0.6500	1.49	15.15	0.056	81.81
Chiapas SS	0.0011	0.0009–0.0012	0.0003	0.0027	3.30	16.00	0.042	1.00
F ₁ RS ^d	0.0040	0.0033–0.0052	0.0019	0.0120	2.70	44.18	0.000	3.63
F ₁ SR ^e	0.0031	0.0022–0.0042	0.0020	0.0120	2.10	148.4	0.000	2.81
F ₂ RS × RS ^f	0.0047	0.0043–0.0053	0.0010	0.0180	2.30	14.9	0.061	4.27
F ₂ SR × SR ^g	0.0055	0.0044–0.0073	0.0029	0.0320	1.68	53.6	0.000	4.50

^aLevel of concentration in micrograms per gram that kills 50 or 99% of larvae.

^b(Upper limit–lower limit) of CL₉₅.

^cLC₅₀ for each strain divided by LC₅₀ for Susceptible (SS) Chiapas strain.

^dF₁ progeny of mass crosses between 203 males RR with 414 females SS (2.06:1 rate) (RS).

^eF₁ progeny of mass crosses between 193 males SS with 388 females RR (2.01:1 rate) (SR).

^fF₂ progeny of mass crosses between F₁ RS males with F₁ RS females.

^gF₂ progeny of mass crosses between F₁ SR males with F₁ SR females.

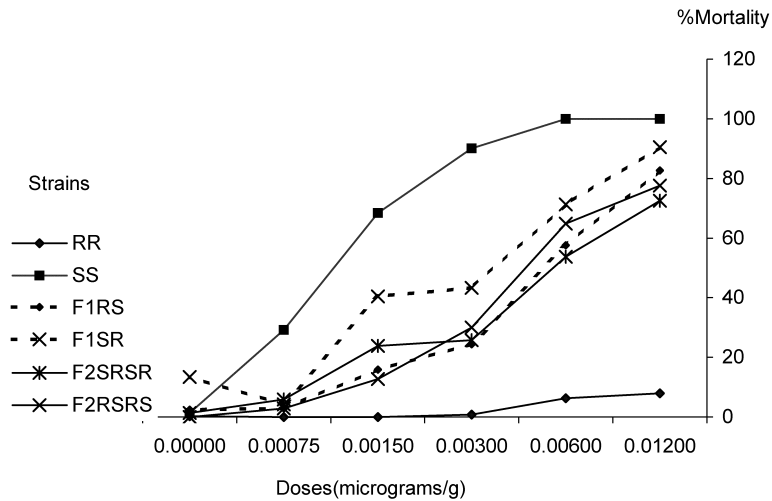


Figure 2. Dose-mortality untransformed curves of the different Aldama *B. microplus* strains tested with flumethrin. RR: Resistant Aldama Strain; SS: Susceptible Chiapas Strain; F₁RS: F₁ progeny of mass crosses between 203 males RR with 414 females SS (2.06:1 rate) (RS); F₁SR: F₁ progeny of mass crosses between 193 males SS with 388 females RR (2.01:1 rate) (SR); F₂SRSR: F₂ progeny of mass crosses between F₁ SR males with F₁ SR females; F₂RSRS: F₂ progeny of mass crosses between F₁ RS males with F₁ RS females.

Table 4. Mortality (%) in the *B. microplus* Aldama resistant (RR), Susceptible (SS) Chiapas and their F₁ hybrids strains and effective dominance levels (D_{ML}) for each dose.

Doses (μ g)	Mortality %			$N(F_1)$	D_{ML}^b
	Aldama RR	Chiapas SS	F ₁ ^a		
0.01200	8.02	100	87.30	821	0.138
0.00600	6.31	100	68.10	830	0.341
0.00300	0.84	90.06	36.60	933	0.599
0.00150	0.00	68.43	27.80	907	0.594
0.00075	0.00	29.94	3.70	758	0.874
0.00000	0.25	1.33	9.70	723	

^aF₁ pooled progeny of reciprocal crosses between RR and SS.

^bEffective dominance $D_{ML} = (ML_{RS} - ML_{SS}) / (ML_{RR} - ML_{SS})$; ML are mortality levels for the resistant homocygotes, heterocygotes, and susceptible homocygotes, respectively.

significant ($P < 0.01$) higher weights than resistant females and their crosses (SR). Maternal effects are thus shown for egg-mass. The lower hatchability percentage was seen in the SR strain, furthermore in this strain, only 16 of 24 egg-masses also hatched (Table 4).

Discussion

Results of this study indicated that inheritance for flumethrin resistance in the Aldama strain was autosomal and controlled by more than one gene.

The Aldama *B. microplus* strain has the same pattern of resistance as the characterized Lamington strain in Australia, with specific resistance to flumethrin (Nolan 1989). The single-gene hypothesis was rejected, except for the 0.006 dose. These results are in broad agreement with the work of Stone and Youton (1982) with two Australian populations of *B. microplus*, one resistant to diazinon and other to chlorpyrifos. They reported that resistance was due to two complementary genes that jointly exhibited incomplete dominance.

In the present study the one gene hypothesis was not rejected at the 0.006 µg/g dose, which is surprising because at 0.012 µg/g the hypothesis was rejected. This confirms the complexity of the resistance mechanisms in this strain of *B. microplus*. It could be gene interactions having effects in opposite directions, canceling the individual effects (Lynch and Walsh 1998).

Resistance mechanisms in *B. microplus* to pyrethroids appears to be multifactorial, and it varies among strains (Miller et al. 1999; Jamroz et al. 2000, Guerrero et al. 2001) suggesting that resistance arose independently in these populations (Tabashnik et al. 1997) with different genetic solutions (French-Constant et al. 1993). This was reported in *Lucilia cuprina* (McKenzie et al. 1992) whose resistance selection response to the insecticide diazinon was single-gene based when a high dose was used for selection; however when selection was made with a low dose, the resistance response was polygenically based.

Groeters and Tabashnik (2000) suggested that many genes affect resistance, but that the distribution of effects across loci is not uniform. One or a few loci may often account for most of the resistance. If more than one gene occurs, epistasis can occur as a consequence of increased or decreased enzyme activities (Lynch 1998).

Sex linkage

The results of bioassays following reciprocal mass crosses between the Aldama and Chiapas strains showed that there was no significant difference ($P > .05$) in the LC_{50} or slope between the F_1 progeny thus eliminating the possibility of sex linkage and indicating that the resistance to flumethrin in the Aldama *B. microplus* resistant strain was autosomally inherited. This was shown in previous works of resistance to the *B. thuringiensis* toxin in *Heliotis virescens* (Gould et al. 1997), *P. xylostella* (Tabashnik et al. 1997; Sayyed et al. 2000) and *Pectinophora gossypiella* (Liu et al. 2001).

Resistance factors

The resistance factor found for the Aldama *B. microplus* strain (81.81) was different than those found for Coatzacoalcos (15.7), Corrales ($> 24,300$) and San Felipe

Table 5. Average egg-mass weight and hatchability percentage mean for females *B. microplus* susceptible, resistant strains and their reciprocal crosses.

Strain	Egg-mass (g) weight mean (n)	Percentage hatchability mean ^a (n)
Aldama RR	0.148 ^b (48)	75.5 ^b (24)
Chiapas SS	0.195 ^c (48)	79.0 ^b (24)
RS ^d	0.184 ^c (48)	60.6 ^{b,c} (23)
SR ^e	0.165 ^b (48)	43.3 ^c (16)

^aHatchability percentage was transformed to arcsin \sqrt{p} to perform the analysis of variance.

^{b,c}One-way ANOVA with Tukey test of means was used. Literal different they denote significant differences $P < 0.05$.

^dF₁ progeny of mass crosses between 203 males RR with 414 females SS (2.06:1 rate) (RS).

^eF₁ progeny of mass crosses between 193 males SS with 388 females RR (2.01:1 rate) (SR).

(> 24,300), Mexican *B. microplus* strains treated with flumethrin after 15 generations of selection with permethrin in the study of Miller et al. (1999). This resistance factor was higher than those obtained by seven selection generations of a *Boophilus microplus* strain for resistance to permethrin (20.9) (Davey and George 1998). F₁ and F₂ larvae had similar lower resistance factors (Table 5) while the resistant Aldama strain was 21-fold higher than the average of F₁ and F₂ resistance factor. The lower values of resistance factors in F₁ hybrids compared with the resistant strains, was also seen in the Stone and Youlton (1982) study.

The resistance factor was calculated as the LC₅₀ of one population divided by the LC₅₀ of the most susceptible population. Regardless of the LC₅₀ these practices assume that the values for each group has been estimated with equal precision (Robertson et al. 1991). In the RR Aldama strain, this was not true because the width of its 95% confidence limits (0.556) was higher than those of the susceptible Chiapas strain (0.0003). Therefore, this resistance factor has to be taken with caution.

Dominance of resistance

Effective dominance was obtained for resistance to flumethrin in the Aldama *B. microplus* strain. The effective dominance assesses the relative ML for a given insecticide concentration, and the LC₅₀ precision problem does not affect this value. In practice it is a better choice than those using LC₅₀ such as the Stone (1982) study which is of little value for pest resistance management because strategies for delaying insecticide resistance mostly involve determining whether it is technically feasible to use doses such that all heterozygotes are killed (Curtis et al. 1978). In the present study, the extent of flumethrin resistance in the Aldama *B. microplus* strain depended upon the concentration of the pesticide used. Resistance was almost dominant at the lowest dose while almost completely recessive at the highest dose (Table 3). But in the commercial dose, resistance was additive. Similar results were reported in *P. xilostella* resistance against *B. thuringiensis* toxins

(Sayyed et al. 2000, Liu et al. 2001). These results support the existence of at least two different genes (Sayyed et al. 2000). The degree of dominance affects the strategies for managing insecticide resistance, a high-dose strategy can slow resistance but only when the alleles exist in the population as heterozygotes (Tang et al. 1997). The dose-dependence of dominance resistance to flumethrin in the Aldama *B. microplus* strain may indicate that a high concentration of the pesticide is essential to kill hybrid F₁ offspring in a high-dose and refuge strategy.

Reproductive variables

Egg-mass showed a maternal effect in this trial. Lower egg-mass in the Aldama resistant strain and their resistant female F₁ cross (SR), could be due to the lack of fitness in the resistant females that might spend more energy to survive than the others (Bennet 1975). The same occurred for the egg-mass viability of the SR strain compared with the others: (16/24 vs. 24/24), and had 36% less hatchability than the susceptible Chiapas strain.

The observed variation in the genetic basis of resistance to ixodocides in *B. microplus*, profoundly affected the choice of strategies for combating it. In Mexico, integrated resistant management is needed to slow the development of resistance (Miller et al. 1999).

Our results shown here indicated that more than one gene of flumethrin resistance in *B. microplus* tick are present. Therefore is necessary to locate and understand the major loci for elucidate the mechanism of resistance and improve the ability to track and delay the evolution of resistance.

References

- Anonymous. 1971. Recommended methods for detection and measurement of resistance of agricultural pests to pesticides: tentative method for larvae of cattle ticks, *Boophilus* spp. FAO Meth. 7(19): 15–18.
- Bourguet D., Genissel A. and Raymond M. 2000. Insecticide resistance and dominance levels. J. Econ. Entomol. 93: 1588–1595.
- Brogdon W.G. and McAllister J.C. 1998. Insecticide resistance and vector control. Emerg. Infect. Dis. 4: 605–612.
- Cochrane B.J., Windelspecht M., Brandon S., Morrow M. and Dryden L. 1998. Use of recombinant inbred lines for the investigation of insecticide resistance and cross resistance in *Drosophila simulans*. Pest. Biochem. Physiol. 61: 95–114.
- Collins R.L. 1967. A general nonparametric theory of genetic analysis. I. Application to the classical cross. Genetics 56: 169–170.
- Curtis C.F., Cook L.M. and Wood R.J. 1978. Selection for and against insecticide resistance and possible methods of inhibiting the evolution of resistance in mosquitoes. Ecol. Entomol. 3: 273–287.
- Davey R.B. and George J.E. 1998. *In vitro* and *in vivo* evaluations of a strain of *Boophilus microplus* (Acari: Ixodidae) selected for resistance to permethrin. J. Med. Entomol. 35: 1013–1019.
- De Campos P.M. 1998. Daily mean number of eggs laid by the southern cattle tick (Acari: Ixodidae) compared with mean egg mass weight. J. Econ. Entomol. 91: 153–158.
- Estrada-Peña A. 1999. Geostatistics and remote sensing using NOAA-AVHRR satellite imagery as predictive tools in tick distribution and habitat suitability estimations for *Boophilus microplus* in South America. Vet. Parasitol. 81: 73–82.

- French-Constant R.H., Steichen J., Rocheleau T.A., Aronstein K. and Roush R.T. 1993. A single amino acid substitution in a γ -aminobutyric acid subtype A receptor locus associated with cyclodiene insecticide resistance in *Drosophila* populations. *Proc. Natl. Acad. Sci. USA* 90: 1957–1961.
- Finney D.J. 1964. *Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve*. 2nd edn. Cambridge University Press, London.
- Guerrero F.D., Davey R.B. and Miller R.J. 2001. Use of an allele-specific polymerase chain reaction assay to genotype pyrethroid resistant strains of *Boophilus microplus* (Acari: Ixodidae). *J. Med. Entomol.* 38: 44–50.
- Gould F., Anderson A., Jones A., Sumerford D., Heckel D.G., Lopez J., Micinski S., Leonard R. and Laster M. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. *Proc. Natl. Acad. Sci. USA* 94: 3519–3523.
- Groeters F.R. and Tabashnik B.E. 2000. Roles of selection intensity, major genes, and minor genes in evolution of insecticide resistance. *J. Econ. Entomol.* 93: 1580–1587.
- He H., Chen A., Davey B.R., Ivie W.G., Wagner G.G. and George E.J. 1999. Sequence analysis of the knockdown resistance-homologous region of the para-type sodium channel gene from pyrethroid-resistance *Boophilus microplus* (Acari: Ixodidae). *J. Med. Entomol.* 36: 539–543.
- Jamroz R.C., Guerrero F.D., Pruett J.H., Oehler D.D. and Miller R.J. 2000. Molecular and biochemical survey of acaricide resistance mechanisms in larvae from Mexican strains of the southern cattle tick, *Boophilus microplus*. *J. Insect Physiol.* 46: 685–695.
- Jonsson N., Mayer D.G., Matschoss A.L., Green P.E. and Ansell J. 1998. Production effects of cattle tick (*Boophilus microplus*) infestation of high yielding dairy cows. *Vet. Parasitol.* 78: 65–77.
- Liu Y.B., Tabashnik B.E., Meyer S.K., Carriere Y. and Bartlett A.C. 2001. Genetics of pink bollworm resistance to *Bacillus thuringiensis* toxin Cry1Ac. *J. Econ. Entomol.* 94: 248–252.
- Lynch M. and Walsh B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates Inc., Canada.
- McKenzie J.A., Parker A.G. and Yen J.L. 1992. Polygenic and single gene responses to selection for resistance to diazinon in *Lucilia cuprina*. *Genetics* 130: 613–620.
- Miller R.J., Davey R.B. and George J. 1999. Characterization of pyrethroid resistance and susceptibility to coumaphos in Mexican *Boophilus microplus* (Acari: Ixodidae). *J. Med. Entomol.* 36: 533–538.
- Montgomery D.C. 1991. *Diseño y Analisis de Experimentos*. Grupo Editorial Iberoamerica, México.
- National Research Council. 1986. *Pesticide Resistance: Strategies and Tactics for Management*. National Academic Press, Washington, DC.
- Nolan J., Wilson J.T., Green P.E. and Bird P.E. 1989. Synthetic pyrethroid resistance in field samples in the cattle tick (*Boophilus microplus*). *Aust. Vet. J.* 66: 179–182.
- Robertson J.L. and Preisler H.K. 1991. *Pesticide Bioassays with Arthropods*. CRC Press, BocaRaton, U.S.A.
- Santamaría E.M. and Fragoso S.H. 1994. Resistencia en garrapatas *Boophilus microplus* a los ixodicidas en México. In: *Proc. XIV Pan Am. Cong. Vet. Sci.*, 9–15 October 1994, Acapulco, Guerrero, México, pp. 473–474.
- Sayyed A.H., Haward R., Herrero S., Ferré S. and Wright D.J. 2000. Genetic and biochemical approach for characterization of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a field population of the diamondback moth, *Plutella xylostella*. *Appl. Environ. Microbiol.* 66(4): 1509–1516.
- Solomon K.R. 1983. Acaricide Resistance in Ticks. *Advances in Veterinary Science and Comparative Medicine*. Vol. 27. Academic Press, New York, USA. pp. 273–294.
- Stone B.F. and Haydock K.P. 1962. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Canestrini). *Bull. Entomol. Res.* 53: 563–578.
- Stone B.F. and Youlton N.J. 1982. Inheritance of resistance to chlorpyrifos in the Mt Alford strain and to diazinon in the Gracemere strain of the cattle tick (*Boophilus microplus*). *Aust. J. Biol. Sci.* 35: 427–440.
- Tabashnik B.E., Liu Y.-B., Malvar T., Heckel D.G., Masson L., Ballester V., Granero F., Ménsua J.L. and Ferré J. 1997. Global variation in the genetic and biochemical basis of diamondback moth resistance to *Bacillus thuringiensis*. *Proc. Natl. Acad. Sci. USA* 94: 12780–12785.

- Tang J.D., Gilboa D.S., Roush R.T. and Shelton A.M. 1997. Inheritance stability, and lack-of-fitness cost of field selected resistance to *Bacillus thuringiensis* in diamondback moth (*Plutella xylostella*) (Lepidoptera:plutellidae) from Florida. J. Econ. Entomol. 90: 732–741.
- Throne J.E., Weaver D.K. and Baker J.E. 1995. Probit analysis: assessing goodness-of-fit based on backtransformation and residuals. J. Econ. Entomol. 88: 1513–1516.